European Journal of Immunology

Supporting Information

for

DOI 10.1002/eji.201040649

Interferon-α induces unabated production of short-lived plasma cells in pre-autoimmune lupus-prone (NZB × NZW)F1 mice but not in BALB/c mice

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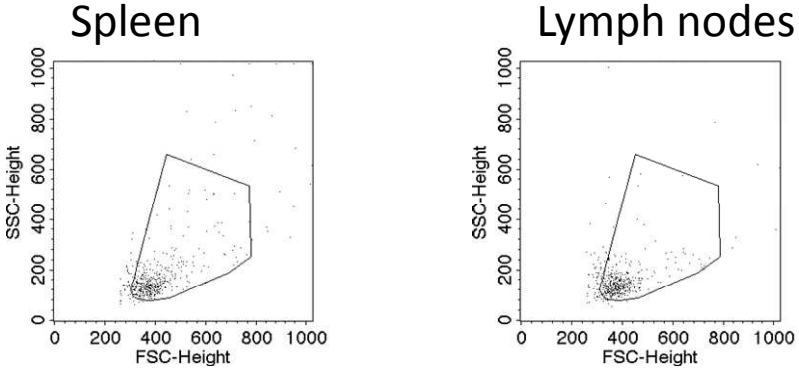
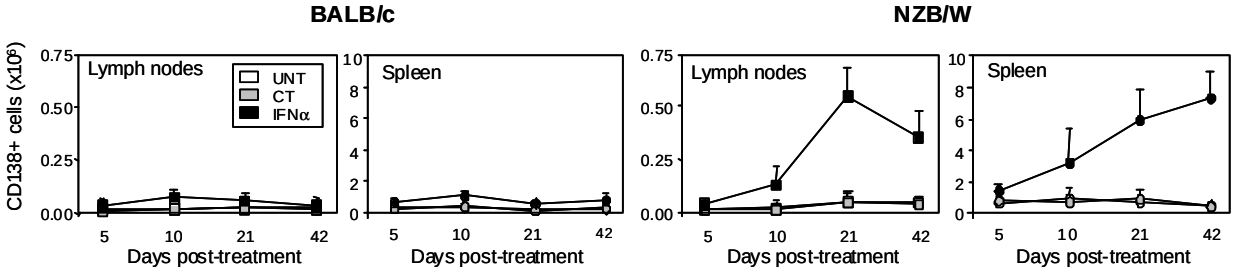
Supporting Information

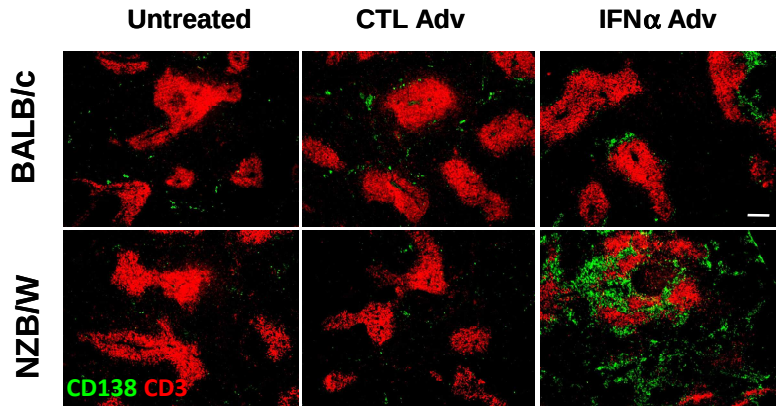


Supplemental Figure 1. Gates used to analyze spleen and lymph nodes cells by FACS



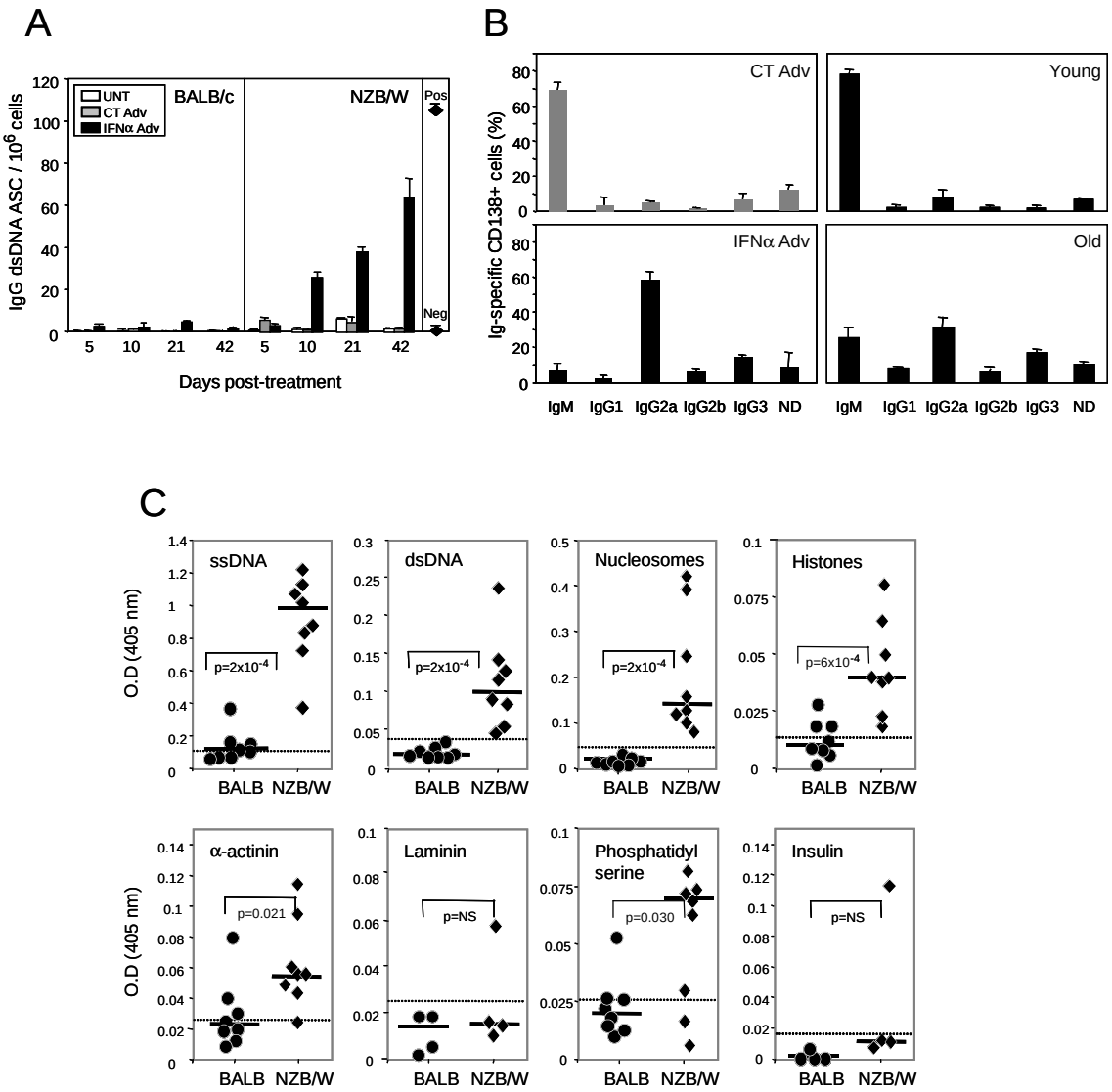
Supplemental Figure 2. Number of $B220^{lo}CD138^{+}$ cells in spleens and lymph nodes of NZB/W and BALB/c mice

FACS with an antibody to CD138, a marker of ASCs, was used to examine the effect of IFNα on the numbers of $CD138^{+}$ cells in the spleen and in lymph nodes of NZB/W and BALB/c mice. Spleen and lymph node cells from mice sacrificed at indicated times after initiation of treatment were stained with fluorescent Abs, and absolute numbers of $B220^{lo}CD138^{+}$ cells determined by multiplying the frequency obtained by FACS by the spleen absolute cell numbers. Results are the means ± SD of 3 to 5 independent experiments. Expression of IFNα caused a dramatic increase in the absolute number of CD138-positive cells in both tissues in NZB/W but not BALB/c mice. UNT, untreated; CT, CT Adv-treated; INFα, INFα Adv-treated.

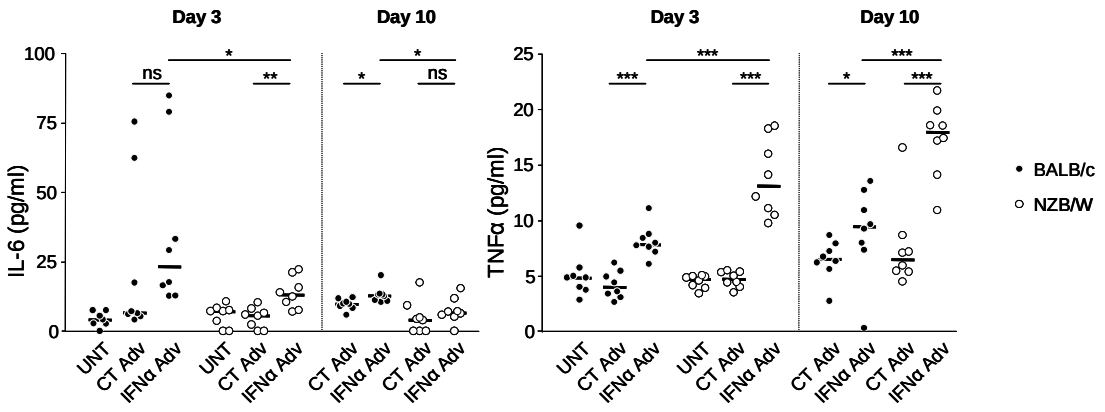


Supplemental Figure 3. High numbers of CD138$^+$ cells are localized in the spleens of IFNα-treated NZB/W mice

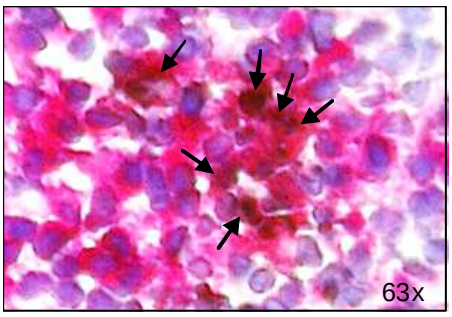
Immunofluorescent staining of plasma cells on spleen sections from IFNα-treated NZB/W mice for CD138 (green) and CD3 (red). At day 23 post-treatment, high numbers of CD138$^+$ plasma cells were detected in the spleen sections of IFNα-treated NZB/W mice but not in control mice. Note that CD138$^+$ plasma cells were scattered in both the red pulp and in the white pulp within the T-cell zone. This was not observed in the spleens of control mice or in the spleens of control or IFNα Adv-treated BALB/c mice. Bars, 100 µm (magnification, x10).



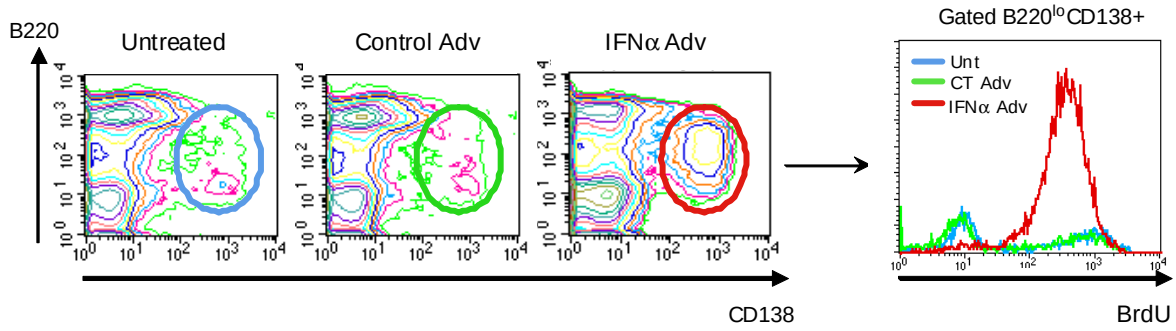
Supplemental Figure 4. Characteristics of IFNα-induced ASCs.
(A) Kinetics of spleen IgG anti-dsDNA ASC production as determined by ELISPOT. The right panel shows the frequency of IgG anti-dsDNA ASCs in the spleen of young untreated NZB/W mice (neg) and old, proteinuric untreated NZB/W mice (pos). Results are the mean ± SD of pools of 3 mice in each group in two independent experiments. (B) Pattern of intracellular Ig class and sub-class in $B220^{lo}CD138^{+}$-gated spleen cells from CT Adv- or IFNα Adv-treated NZB/W young and old, proteinuric NZB/W mice. Cells were stained intracellularly with fluorescent anti-Ig Abs, and the frequency for Ig class and subclass was analyzed by FACS. ND indicates $B220^{lo}CD138^{+}$ cells negative for the studied Abs. Results are the means ± SD of 3 mice per experimental group. (C) IFNα-induced autoantibody reactivity in serum from BALB/c and NZB/W mice was analyzed by ELISA at day 21 post-treatment. Each dot represents an individual mouse. Solid lines represent the median OD. Dashed lines represent the OD value beyond which mice are considered positive (mean OD + 3 SD of sera from CT Adv-treated NZB/W and BALB/c mice).



Supplemental Figure 5. In vivo Adv-mediated delivery of mIFNα induces expression of IL-6 and TNF-α in the sera of Balb/c and NZB/W mice.
Ten-week-old BALB/c (filled) and NZB/W mice (open) were injected with 1 x 10^{10} control-Adv or IFNα-Adv viral particles. Mice were bled at days 3 and 10 post-treatment, and IL-6 and TNF-α serum levels were measured by ELISA. Each dot represents an individual mouse, and lines show median values. Comparisons were made using the Mann-Whitney U test. ns, not significant; *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.



Supplemental Figure 6. In situ apoptosis of spleen cells
Spleen sections of IFNα-treated NZB/W mice were stained for apoptotic $CD138^+$ cells using TUNEL at day 48 post-treatment. TUNEL-stained nuclei appear brown, and $CD138^+$ cells appear magenta. Numerous apoptotic $CD138^+$ cells were present (arrows). At day 48 after initiation of IFNα treatment, 7.2 ± 3.7% of splenic $CD138^+$ cells were apoptotic (vs. 2.6 ± 2.0% in CT Adv-treated mice; data not shown). Results are representative of 6 individual mice.



Supplemental Figure 7. FACS of plasma cells.
Untreated, CT Adv-treated, or IFNα Adv-treated NZB/W mice (at day 0) were fed BrdU for different 14-day periods at the end of which mice were sacrificed and spleen cells analyzed by flow-cytometry. Spleen cells were stained for intracellular BrdU, and BrdU staining of gated $B220^{lo}$ $CD138^{+}$ cells was analyzed by flow-cytometry as illustrated.